*H-Y Antibody Results in Male Patients with Female Donors*

| | Any H-Y Antibody | 2 or more H-Y Antibodies |
|---|---|---|
| TCD HSCT + prophylactic CD8 depleted DLI | 6/6 100% | 4/6 67% |
| TCD HSCT alone | 3/9 33% | 1/9 11% |
| CD8 depleted DLI for relapse | 9/12 75% | 9/12 75% |
| Unmanipulated DLI for relapse | 8/12 67% | 2/12 17% |

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### IN VIVO TRAFFICKING OF CD4+CD25+ REGULATORY T-CELLS IN ALLOGENEIC RECIPIENTS USING BIOLUMINESCENCE IMAGING

*Nguyen, V.H.; Wieland, C.; Contag, C.; Negrin, R. Stanford University School of Medicine, Stanford, CA.*

CD4+CD25+ regulatory T-cells (Treg) have the potential to suppress aberrant immune responses and to regulate peripheral T-cell homeostasis. In a murine allogeneic bone marrow transplantation (BMT) model, we previously showed that Treg suppress graft-versus-host-disease (GVHD) without abrogating the beneficial graft-versus-tumor immunological effect. In the current study, we investigate the in vivo trafficking of Treg to better understand how localization may affect their regulatory function. We have developed and characterized a transgenic mouse which constitutively expresses the luciferase gene in all hematologic cells. Treg from the spleen and lymph nodes of luc+ transgenic FVB/N (H-$2^q$) mice were cotransplanted into lethally-irradiated Balb/c (H-$2^d$) host along with wild-type FVB/N T-cell-depleted bone marrow (TCD-BM) cells and whole splenocytes, the latter containing approximately 30% T cells, which induce GVHD. Bioluminescence imaging (BLI) was performed at various time points. Within the first 48 hours, Treg localized to the cervical lymph nodes (LN) and the spleen. By day 3, signal is detected in other LN (axillary, mesenteric, inguinal) as well as Peyer's patches and liver. Signal intensity, measured by photons/second/mouse, significantly increased and peaked on day 4, consistent with the migration and proliferation of Treg to and at these secondary lymphoid organs, respectively. Skin infiltration of Treg is noted on day 6, congruent with a decreased intensity in the spleen, liver, and lymph nodes. A similar pattern of early migration and proliferation of effector immune cells is noted in the GVHD control group, which is transplanted with wild-type FVB/N TCD-BM and luc+ FVB/N whole splenocytes. However, with the GVHD group, the signal intensity continues to increase at all sites. Continued BLI of the Treg group up to day 45 demonstrates persistent strong signal in lymphoid organs and skin sites. Clinically, the Treg group had no significant evidence of GVHD. Chimerism studies on day 45 show complete donor origin, however, lymphoid reconstitution of CD4+ and CD8+ T cells is delayed in the GVHD control group and enhanced in the recipients transplanted with Treg. The aforementioned results indicate that in vivo, Treg proliferate and survive long-term. In addition, they colocalize with effector immune cells to secondary lymphoid tissues to positively impact clinical outcomes and lymphoid reconstitution following major MHC-mismatched BMT.

# HEMATOPOIESIS/MESENCHYMAL CELLS

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### BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AUGMENT TISSUE ENGINEERED ENTEROCYTES

*Gandia, C.; Yu, H.; Santiago, S.; Coats, E.; Defaria, W.; Ruiz, P.; Tzakis, A.; Kleiner, G.I. University of Miami School of Medicine, Miami, FL.*

Bone marrow-derived MSCs have been demonstrated to have multipotential differentiating ability. Despite recent advances in in vitro models, a reliable method of expansion and long-term maintenance of normal intestinal epithelial cells (IECs) is lacking. Most of the limited data arise from studies based on T84 and Caco-2 colon carcinoma lines. We evaluated a novel protocol of isolation and long-term culture of IEC from rodent enterocytes. The ability of MSC to augment tissue engineered small bowel was evaluated. **Methods:** Small intestine from neonatal DA rats (12 days old) was harvested and digested using cold and warm solution of dispase/collagenase. Primary culture cells in minimal media with high glutamine were initiated. No growth factors were used. Confirmation of epithelial and endothelial cells in the primary culture was verified by Immunohistochemical markers (cytokeratin 19, 5, and 8; anti-cytokeratin+ basal monoclonal antibody; MadCAM-1; and antilaminin B2). Long-term cultures (> 100 days) were obtained. Cytokine secretion panels were determined by BioPlex assay and intestinal metabolites (citrulline, praline, glutamine, lactate) by GC/mass spectroscopy. Bone marrow-derived MSCs were added to cultures. **Results:** Primary culture tissue expressed intestinal epithelial and endothelial. Cytokeratin 19 was the most abundant, followed by cytokeratin 5 and 8, MadCAM-1, anticytokeratin+ basal monoclonal antibody, and laminin B12 (in descending order). Primary and long-term (> 100 days) cultures secreted citrulline, a specific marker of functional enterocytes. Addition of bone marrow-derived MSCs significantly augmented citrulline production ($P < .05$). Cytokines TNF alpha, IL-10, and GMCSF correlated with citrulline levels. The results suggest that bone marrow-derived MSCs augment intestinal development in an in vitro rodent model and may be useful in tissue engineering applications.

# HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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### PARTICULAR HLA-DPB1 ALLELE MISMATCHES PREDICT FOR WORSE OVERALL SURVIVAL IN RECIPIENTS OF UNRELATED DONOR HAEMATOPOIETIC STEM CELL TRANSPLANTS

*Shaw, B.E.; Marsh, S.G.E.; Mayor, N.P.; Madrigal, A. The Anthony Nolan Research Institute, Royal Free Hospital, Hampstead, London, United Kingdom.*

In the HLA-DPB1 molecule there are 6 hypervariable regions (HVR), A to F, in exon 2, which encode for the ββ domain and form the peptide binding groove (PB). Within each of these HVRs are 1 or more amino acids that are polymorphic, and this polymorphism is important in determining the peptides that will bind the recognition by T-cell receptors or both. Mismatches at this level may predict for transplant complications, and this analysis may contribute to a better understanding of the function of DPB1. We analyzed the outcome in 282 mixed transplant pairs who received an hematopoietic stem cell transplant (HSCT) from an unrelated donor. All of the pairs were matched at the allelic level for class I and II. Transplant pairs were assessed as matched or mismatched for HVRs A to F, in a graft-versus-host direction. Mismatches at both amino acid position 65 (within HVR D) and 57 (within HVR C) were associated with transplant complications. Position 65 was matched in 231 (82%) pairs and mismatched in 51 (18%). Matched pairs had a significantly improved overall survival (OS) compared with mismatched pairs (49% vs 35%; log rank $P = .039$). Position 57 was matched in 233 pairs (83%) and mismatched in 49 (17%). There was no significant association between matching at either position and graft-versus-host disease or disease relapse. In contrast, mismatched pairs had a significantly increased rate of transplant-related mortality (TRM). In conclusion, we have shown a significantly worse OS, largely mediated by an increased TRM, in recipients of HSCT from UD which are mismatched at amino acid positions 57 and 65 of the DPB1 molecule.